HYDROPHILIC-LYPOPHILIC BALANCE OF *Xylopia ochrantha* ESSENCIAL OIL NANOEMULSION AND LARVICIDAL ACTIVITY AGAINST *Aedes aegypti*

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Introduction

The future of humanity depends on sustainability. The World Health Organization has a global concern to improve the control of vectors that are responsible for recent outbreaks of diseases such as dengue that is transmitted by the female *A. aegypti* mosquito. According to the Oswaldo Cruz Foundation[1], the best way to fight mosquitoes is during the aquatic phase (larvae and pupae). The larval stage consists of feeding and growth, which in good conditions does not exceed more than five days. However, another combat strategy against the winged phase is with the use of insecticides which proved ultimately to be limited due to the resistance developed to these products by the vector's natural populations[2]. Thus compounds of plant origin appear to be an environmentally safe and promising alternative. Plant species are important sources of bioactive substances and have activity against insects due to their different chemical structures. Restinga de Jurubatiba National Park is part of the Atlantic Forest domain and is considered as an integral protection unit. There is an extensive knowledge gap in this ecosystem which is home to many plant species of interest in scientific research due to their potential biological use. In this environment, some native species had the chemical constituents identified in their essential oils and showed proven bioactivity. The formulation of an innovative and safe nanoemulsion from *Xylopia ochrantha*, one of the restinga flora's species, shows insecticidal potential. The objective of this work is to prepare a stable nanoemulsion from the essential oil of *X. ochrantha* leaves and determine its hydrophilic-lypophilic balance (HLB) and larvicidal activity against L3 larvae of *A. aegypti*.

Material and Methods

Different nanoemulsions were prepared using the low-energy method, containing 5% of the oil phase, 5% of surfactants (Span 80 and Tween 20), and 90% of the aqueous phase. The oil phase and surfactants were kept under mechanical agitation (400 rpm) for 30 minutes. Then, the aqueous phase was added to the oil phase and kept under stirring for another 60 minutes. Several nanoemulsions were prepared containing different proportions of surfactants in the HLB range between 4.3 and 16.7. The experimental protocol for the larvicide assay was carried out in accordance with the WHO[3]. *A. aegypti* larvae were obtained from the Insect Biology Laboratory (Universidade Federal Fluminense, Brazil). All experiments were performed in triplicate with 10 L3 stage larvae in each replicate (n = 30). The optimized nanoemulsion of *X. ochrantha* was diluted in distilled water at 400, 300, 200 and 100 ppm. The negative control group was treated with deionized water. The positive control group was treated with a nanoemulsion without any essential oil. The levels of mortality were recorded after 24 and 48 hours of exposure. Probit analysis was performed with a 95% confidence interval.

Results and Discussion

The choice of the best formulation of nanoemulsion was based on droplet size and index values

of polydispersion, since these characteristics can indicate stability. The nanoemulsion that presented the smallest droplet size (74.56 nm) and the best polydispersion index (0.271) was made with 70% of Tween 20 and 30% of Span 80 and the EO HLB was 14.22. Araújo et al.[4] also working with *X. ochrantha* reported a different value for the nanoemultion which had an HLB at 9.26. Their droplet size was larger (114.0 nm) than that of the present study indicating less stability in the preparation. The larvicide tests of instar L3 showed in 24 hours a mortality rate of 46,67% at the concentration of 400 ppm, 26,67% at 300 ppm, 30,00% at 200 ppm and 20,00% at 100 ppm. The negative control had 0% mortality, as well as the positive control. After 48 hours of experiment, the mortality rate was 93,33% at 400 ppm, 66,67% at 300 ppm, 63,33% at 200 ppm and the 26,67% at 100 ppm. The negative control had 0.00% mortality and the positive control had 16.67%. When the concentration of the nanoemulsion is increased, a higher mortality of larvae occurred over time. In the L3 stage the LC50 was 217,685 ppm and the LC90 was 459,716 ppm within 48 hours. On the other hand, a study verified the larvicidal action of *X. laevigata* and *X. frutecens* against *A. aegypti* (L3) and it was concluded that the genus *Xylopia* did not show larvicidal activity at concentrations below 1000 ppm[5].

Conclusion

It was concluded that the nanoemulsion of *X. ochrantha* leaf essential oil showed lethality at the concentrations tested against L3 instar of *A. aegypti*. Furthermore, this type of research values biodiversity and gives visibility to this important ecosystem, which is the restinga. Thus, it points to the possibility of developing eco-friendly products to solve some public health problems.

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